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# Pulp Cell Duplicating Time in Molars of 60-Day-Old Rats

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# PULP CELL DUPLICATING TIME IN MOLARS OF 60-DAY-OLD RATS

BY

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DEDICATED TO MY MOTHER, FATHER

AND

MY OLDEST BROTHER

(TAWATCHAI TAIYONG)

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TABLE OF CONTENTS


| CHAPTER | | PAGE |
|---|---|---|
| I. | INTRODUCTION | 1 |
| II. | REVIEW OF THE LITERATURE: | |
| | A. The Metabolism of Tritiated Thymidine: | 3 |
| | 1. Pharmacological Aspects: | 3 |
| | a. General Features | 3 |
| | b. Routes of Administration and Doses | 4 |
| | c. Effects of Radiation on Cells | 6 |
| | 2. Histological Aspects - Mitosis and Labeling | 7 |
| | B. Autoradiographic Studies of Mitosis in Various Tissues: | 9 |
| | 1. In Connective Tissue Studies | 9 |
| | 2. In Pulp Tissue Studies: | 11 |
| | a. Animal Studies | 11 |
| | b. Human Studies | 12 |
| III. | METHODS AND MATERIALS: | |
| | A. Selection of Animal | 13 |
| | B. Experimental Procedure | 14 |
| | C. Specimen Preparations: | 14 |
| | 1. Standard Histological Preparation | 14 |
| | 2. Autoradiographic Procedures | 14 |
| | D. Selection of Specimens | 16 |
| | E. Counts of Radioactive Cells | 16 |
| | F. Counts of Pulp Tissues | 18 |

CHAPTER PAGE

IV. FINDINGS:
A. Mitotic Patterns at Two Hour Intervals: 19
1. Pulp Horn Region ..... 19
2. Pulpal Floor Region ..... 19
3. Root Canal Region ..... 20
4. Apical Root Region ..... 20
B. Total Number of Pulp Cells and Its Relation to the Growth of the Pulp ..... 20
C. Interpretation of the Frequency of Labeled Cells ..... 21

V. DISCUSSION:
A. Radioactive Labeled Cells in Relation to the Growth of the Pulp Tissue ..... 23
B. The Cell Density of the Pulp of 60-Day-Old Rats ..... 25
C. Labeling Index ..... 26
D. Duplicating Time of Pulp Cells ..... 28

VI. SUMMARY AND CONCLUSIONS:
Summary ..... 31
Conclusions ..... 32

VII. REFERENCES ..... 34

VIII. ILLUSTRATIONS:
A. Tables ..... 38-40
B. Graphs ..... 56
C. Drawings ..... 44
..... 46-48
..... 55
D. Photomicrographs ..... 41-43
..... 45
..... 49-54
..... 57-61

# CHAPTER I

# INTRODUCTION

A number of investigators have used autoradiographic techniques in studies of various connective tissues, including the dental pulp of different species.

Because the dental pulp consists of connective tissue which is derived from the mesenchyme, the pulpal structural elements and physiology are very similar to loose connective tissue (James, Schour and Spence 1959; Zerlotti 1964; Orban 1966).

Das (1963) in a tissue culture study, described the cells of the pulpal tissue stroma as similar in morphology to fibroblasts. Because such great similarities exist between the cells and physiology of the pulp and other connective tissues, it seems justified to use and make valid comparisons of autoradiographic analyses of pulp with those of other similar tissues.

Autoradiography is a tool known to be helpful in the study of cell multiplication and has been used to study various connective tissues (Leblond, Messier and Kopriwa 1959; Messier and Leblond 1960; Schultze, Oehlert and Leblond 1960). It has also been used in the study of dental pulp (Messier and Leblond 1960; Hoffman and Gillette 1962; Pinzon, Toto and O'Malley 1966).

Indeed in 1966 Pinzon, Toto and O'Malley studied the dental pulp of rats using tritiated thymidine to evaluate the pattern of pulpal changes due to aging. Pinzon, Kozlov and Burch (1967) corroborated the autoradiographic findings with traditional histologic methods of evaluation.

The problem of this paper is to determine the duplicating time of the cells of the rat molar pulp. Duplicating time can be defined as the amount of time expended for a given sample (the tritiated thymidine labeled cell population) of the rat molar pulp cell population to double in number.

This study will use animals of one age. It is possible, however, that as animals age, the duplicating time may be lengthened. Lengthening of duplicating time with increased age could imply a diminished capability for multiplication of cells. Knowledge from this study may aid in the understanding of the capability for pulp repair in the rat molar and may also be applicable to human pulp as well.

# CHAPTER II

# REVIEW OF THE LITERATURE

A. The Metabolism of Tritiated Thymidine:

1. Pharmacological Aspects:

a. General Features:

Studies involving the use of thymidine for specially labeling the DNA of cells for subsequent autoradiography, should be concerned with the possible fates of the labeled material.

Potter (1959) stated that, the metabolism of natural thymidine was divided into two main categories: (1) Conversion to DNA, (2) Conversion to breakdown products, as in figure 6.

Recently tritiated thymidine has been popular as a tool to investigate cell turnover and the dynamics of human and animal tissues both in vivo and in vitro.

In order to label and identify cells which were synthesizing DNA, thymidine containing tritium, a radioactive substance has been used.

Tritium is the isotope of hydrogen having a half life of 12.26 years, and a disintegration rate of 0.016 per cent a day. The maximum in distance of the radiation of tritium is said to be 8 microns in water or tissue and only 2 microns in photographic emulsion. The average range

of the particle was 1.5 microns. Because of the short range of the particle and also because less than 50% of the particles traveled initially in the direction of the emulsion, the overall efficiency of tritium is low (2% in tissue specimens and 5% in smear preparations, Cronkite et al 1959; Hamilton 1959 and Lajtha 1959).

Thymidine is labeled in the pyrimidine portion (pyrimidine bases) of the DNA molecule (Figure 7) by exchange with the hydrogen bound to the carbon at the position two in the ring. According to the manufacturer (Schwarz Bioresearch Inc., Orangeburg, N. Y.), tritiated thymidine thus prepared does not exchange its activity even in the medium of acid or alkali.

b. Routes of Administration and Doses:

Tritiated thymidine may be given by three routes of administration: intravenously, peritoneally and subcutaneously.

In 1960 Rubini, Cronkite, Bond and Fliedner studied the fate and metabolism of tritiated thymidine in human beings. They found that the radioactivity following intravenous injection of tritiated thymidine saturated blood plasma within one minute and then declined. They also

pointed out that tritiated thymidine lost its maximal activity in one minute following injection. Then after injection for five minutes labeled cells could be observed.

Skongaard (1964) found that in marmosets, following intravenous injections of tritiated thymidine, the blood level reached maximal tritiated activity rapidly, and then dropped rapidly. However, the plasma clearance following intraperitoneal and intramuscular injections appeared slower. In addition, he also pointed out that both the tritiated thymidine present at two hours after injection, and the number of grains (silver grains) increase corresponding to the time the tracer (tritium) is available in the blood plasma. However, Potter had earlier (1959) stated that labeling of DNA was completed within one hour in most tissues with a single dose of tritiated thymidine.

Cronkite (1959) found that the disappearance of nonvolatile tritium activity was very rapid. It was estimated that about 10 to 20 percent was lost from the blood plasma in the first circulation. Therefore, he believed that the effective labeling time of tritiated thymidine was less than sixty minutes. During that time it was either degraded or incorporated into newly formed DNA.

In 1958, Painter, Drew and Hughes evaluated various doses of tritiated thymidine on mouse spermatogonia. They suggested that the dose should be between 0.5 to 0.1 microcuries per gram of body weight for excellent radioautographic study of cell turnover. They also stated that 1.25 microcuries per millimole of tritiated thymidine is an inhibitory dose for He La cell growth after exposure for 24 hours. However, other investigators (Cronkite, 1959; Hamilton, 1959; Hughes, 1958 and Johnson, 1959) observed no broken chromosomes or delaying of mitosis after doses of 0.5 to 1.0 microcuries per gram weight, indicating no inhibition of growth. The effect of such doses upon several generations of cells has not been investigated.

According to Bendick (1959); Rubini, Cronkite, Bond and Fliedner (1960), the end product of tritiated thymidine catabolism is B- aminoisobutyric acid. This end product was found in urine, and it was analyzed as a nonvolatile part of the tritiated thymidine.

c. Effects of Radiation on Cells:

Hughes (1958), Cronkite, Bond, Fliedner and Rubini (1959) stated that only newly formed DNA was labeled by means of radioactive thymidine. By using radioactive thymidine, the radioactive thymidine

became part of the newly formed DNA and, therefore, it was possible to label DNA. Lajtha and Oliver (1959) emphasized the possible cell damage from radiation. They said that about 90% of the energy given off by disintegration of the radioactive thymidine took place in the nucleus. Cronkite did not see any evidence of radioactive injury in radiosensitive tissue of the mouse, guinea pig, dog or rat with dose up to 1.0 microcuries per gram of body weight; specific activity of tritiated thymidine was 1.9 curies/millimole. He made the suggestion that the useful dose in human being was 0.1 to 0.2 microcuries per gram of body weight, which would give a very good autoradiography after exposure period of 30 to 60 days.

Furthermore, he confirmed that with doses up to 2.0 microcuries of tritiated thymidine per gram of body weight there was no evidence of cytologic effects from radiation, but he suggested that under usual conditions, the dose of tritiated thymidine should be the smallest possible one with normal life expectancy and no immediate damage.

2. Histological Aspects - Mitosis and Labeling:

The nucleic acids of the cell, DNA and RNA, differ in their component nitrogeneous bases. DNA contains the bases cytosine, adenine,

guanine and thymine, while RNA contains cytosine, adenine, guanine and uracil. (Watson and Crick 1953, Figure 7).

It is possible to label DNA but not RNA by using radioactive thymidine, because thymidine is a specific precursor of the thymine of DNA (Friedkin, Tilson and Roberts, 1956). Only newly formed DNA was labeled. This was pointed out by Amano, Messier and Leblond (1959) in their study of tissue of mice. They had prepared the extraction of DNA from histological sections by means of the enzyme deoxyribonuclease. Then they observed the radioautographic reaction over nuclei eight hours after injection of tritiated thymidine.

Hughes (1958) studied DNA synthesis and found no evidence of turnover of DNA. He also stated that a cell that was synthesizing DNA was a cell preparing to divide.

Thompson, Paul and Davids (1958) studied the metabolic stability of nucleic acid in vitro. The experiment was done by growing cells on medium containing a radioactive substance and then transferring them to a nonradioactive medium, so that the loss of radioactivity from DNA could be measured. They found that a small amount of labeled DNA was lost. Therefore, they suggested that there was a possibility of a slight turnover of DNA.

Recent studies confirmed the idea of DNA replication as the basis for the duplication of chromosomes preceding mitosis. Taylor, Woods and Hughes (1957) demonstrated that when chromosomes were labeled by tritiated thymidine and visualized by autoradiography of tissue sections, each daughter chromosome in the following mitosis received half of the labels if mitosis was successful. After a second mitosis, half of the chromosomes were labeled and the other half were completely nonlabeled.

A similar finding was reported by Meselson and Stahl (1958). In labeling the DNA of Escherichia coli by sedimentation techniques with the radioactive 15N showed increased density of the DNA molecule and they affirmed that subsequent growth for one generation resulted in an accumulation of half-labeled DNA molecules. Then after the next cell division, only half-labeled molecules and nonlabeled molecules were obtained.

B. Autoradiographic Studies of Mitosis in Various Tissues:

1. In Connective Tissue Studies:

Autoradiographic techniques have been utilized in studies of the cell proliferation in various types of connective tissues of different animals.

In 1959, Cronkite, Bond, Fliedner and Rubini studied connective tissues of mice and rats. They found labeled cells throughout the body one hour after injection of radioactive thymidine.

Leblond, Messier and Kopriwa (1959) also studied connective tissues of mice and rats. They divided the animals into two groups: (1) using adult mice as the cell formation test group, each animal was injected with a single dose of tritiated thymidine, and then sacrificed eight hours later, (2) using 3-day-old rats as the cell retention test group, the animals were sacrificed six months after injection of the same dosage of tritiated thymidine.

They found that the cell formation test group showed surprisingly high numbers of labeled nuclei scattered in various types of connective tissues. Some areas of the connective tissues showed increased numbers of labeled cells. They implied that this increase resulted from a higher rate of nonlabeled cell loss. In the cell retention test group, they found fewer labeled cells. Therefore, they concluded that the connective tissue cells might consist of a cell population with cellular variation in proliferative potencies. Furthermore, in 1960, Messier and Leblond reported that the rat loose connective tissues showed a moderate

frequency of labeled cells. In some connective tissues such as cartilage or bone, the labeling cells were found at growth centers. This was also found in studying the roots of erupting teeth (Hoffman and Gillette, 1962).

2. In Pulp Tissue Studies:

a. Animal Studies:

In 1960, Messier and Leblond briefly described the labeling reaction in the pulpal tissue of rat molar and incisor teeth. They reported that labeled cells were very seldom seen in the molar pulpal tissue which in many ways was very similar to human dental pulp. The pulp of the rat incisor, however, contained many labeled nuclei of odontoblasts particularly at the site of odontogenesis. Also, in 1962, Hoffman and Gillette confirmed that the mitotic activity of the pulp of an erupting tooth was at the apical areas adjacent to the Hertwig's epithelial diaphragm, root growth center.

Furthermore, in 1966, Pinzon, Toto and O'Malley studied maxillary first molars of albino rats aged 10 to 400 days. All animals were injected intraperitoneally with tritiated thymidine and sacrificed one hour later. These sections were prepared for autoradiographs and studied. They concluded that:

(1) The rat molar pulp showed DNA synthesis which indicated cell division at all ages through 400 days.

(2) The rat molar pulp was capable of cell proliferation at least for a period of 400 days.

(3) The proliferation of cells of the pulp was greater in the younger age groups.

(4) The volume of the pulp was reduced by constant dentin apposition, and a slight relative increase in the cell population was observed (increased cell density).

(5) The connective tissue of the rat molar pulp was similar to other connective tissue.

b. Human Studies:

At present, there have been no studies of human dental pulp by autoradiographic techniques.

A. Selection of Animal:

The rat has been used as an experimental animal for many years, because it is a convenient and inexpensive animal for laboratory investigations. Many investigators have described anatomical and biological similarities between human and rat molars (Berman, 1957; Schour and Massler, 1962; Kindlova, 1963) and, therefore, it is well suited for studies of dental pulp.

In previous investigations, it was found that the pulp of the 60-to-100-day-old rats (approximately equivalent to young adults) had a greater capacity for healing than the younger age group or the older age group (Berman, 1957; Pinzon, Toto and O'Malley, 1966). Therefore, in this present study, the molars of approximately 60-day-old rats are used.

Fifty right maxillary first molars from fifty healthy female Sprague-Dawley rats* were used.

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*Supplied by Abrams Animal Farm, Chicago, Illinois.

B. Experimental Procedure:

The animals were injected intraperitoneally with tritiated thymidine,* specific activity 1.90 curies per millimole. The dosage level was 0.7 microcuries per gram of body weight. At two-hour intervals over a period of one hundred hours, each injected animal was anesthetized with diethyl ether, and sacrificed by decapitation. The head was skinned, split mid-sagitally, and fixed immediately in ten percent buffered formalin solution.

C. Specimen Preparation:

1. Standard Histological Preparation:

The right maxillary molar region of the jaw from each animal was decalcified in formic-citric acid, dehydrated in ascending orders of alcohol (75, 95 and 100%) and embedded in paraffin. Mesiodistal sections, five to six microns thick, were obtained by standard laboratory procedures.

2. Autoradiographic Procedures:

Histologic sections four to five microns thick were mounted with

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*Supplied by Schwarz Bioresearch, Inc., Orangeburg, New York.

gelatin. The sections were deparaffinized just prior to application of the autoradiographic emulsion $NTB_3$. *

Deparaffinized slides (at 43°c) were dipped into melted emulsion $NTB_3$** (at 43°c) in the dark room. When dry, the slides were stored in the refrigerator in light tight black boxes, containing drying agent, lithium chloride (one teaspoonful) for fourteen days exposure period, then developed with Kodak D-19. **

After development, it was possible to see, microscopically, small areas of developed silver grain within the emulsion coating over the tissue (Figure 1).

In order to visualize the nonradioactive portion of the sections, the tissues were stained through the emulsion using Nuclear Fast Red and Indigo Carmine staining. ***

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*Research Division Special Products, Rochester, New York.

**Followed the method of Brookhaven National Laboratory, Upton, New York.

***Micheline Mortreull-Lauglois, Department of Comparative Anatomy and Histology, Faculty of Science, University of Paris, Paris, France.

This staining technique gives better results in the histological visualization than with hematoxylin and eosin. In hematoxylin and eosin, the nuclei stain dark purple. Therefore, it is difficult to differentiate between labeled nuclei and nonlabeled nuclei. With Nuclear Fast Red and Indigo Carmine, the stain results in staining nuclei red purple, erythrocytes red orange. Bone, dentine and collagenous fibers appear as varying shades of bluish green and are easily identified.

D. Selection of Specimens:

Histologic slides selected for detailed analysis were those exhibiting the best central sections through the crown and two buccal roots of the maxillary first molar (Figures 2A, 2B). Five adjacent serial sections on each slide were selected for mitotic counts (25 sections from each animal), and analyzed on the basis of good isotope appearance, i.e., separate silver graining in the emulsion, and the least sectioning artifact and background radiation (Figure 4).

E. Counts of Radioactive Cells:

The present study was concerned with selecting and counting those cells actively engaged in synthesizing DNA prior to cell division. This synthesis is experimentally determined by the presence of silver grains

in the emulsion over those nuclei which have incorporated radioactive thymidine during DNA synthesis.

Counting was aided with a Whipple disk, Number A. 2433, as in the Figure 3, which is a piece of round thin glass having square spacings in it, inserted into the eyepiece of the microscope, at 400 X magnification. Counts were made of cells synthesizing DNA. The labeled cells of the entire pulp tissue were counted in each square under high dry 400 X magnification (Figures 4 and 5).

In order to be counted as a labeled cell, all three of the following characteristics had to be fulfilled:

1. Silver grains in the emulsion must have a nucleus below them.

2. At least three or more grains over a nucleus were necessary for it to be considered labeled.

3. The labeling grains over a nucleus must be the size and shape of typical grains and they must be at the same approximate level of focus within the emulsion as other obviously labeled cell grains (Figure 4).

In this study, odontoblasts, endothelial cells and intravascular blood cells were excluded, because they cannot be considered as part of the

pulp tissue proper and, therefore, their inclusion in the cell counts might change the significance of pulpal cell counts. Their inclusion would produce an unusual variability in counts.

F. Counts of Pulp Tissues:

Pulp cells in each section of 40 sections (15 sections from the 2-hour interval and 25 sections from the 96-hour interval) were counted with aid of a square Whipple disk as mentioned above (Figures 3 and 5). Pulp cells were counted in each square within the entire pulp (Table II). Also, odontoblasts, endothelial cells and intravascular blood cells were excluded, as above.

In this study, a statistical analysis was used to evaluate the significance of the growth of the pulps of 60-day-old rats.

The average number of pulp cells was determined, and in this study the average number of pulp cells was used as 4000 cells instead of 4246 cells.

The ratio of labeled nuclei to nonlabeled nuclei of the pulp at each two-hour interval was calculated (Table I). The purpose of this calculation was to determine at which time the number of labeled pulpal cells doubled.

# CHAPTER IV

# FINDINGS

A. Mitotic Patterns at Two-Hour Intervals:

For purposes of description, the pulp tissue was divided into four different regions: 1. pulp horn, 2. pulp chamber floor, 3. root canal, and 4. apical root regions.

1. Pulp Horn Region:

Two hours after thymidine injection there were a few pulp horn cell nuclei that were radioactive. Radioactivity in the first few hours was seen in the nuclei located near the center of the horn as in Figures 8 and 9. It should be mentioned, however, that during the entire experimental period the location of the radioactive cells appeared to move from the center of the horn to other areas. Some radioactive cells were seen closer to the blood vessel walls within the horn, some appeared near the odontoblastic layers, and some appeared closer to the tip of the horn (Figure 10).

2. Pulpal Floor Region:

Throughout the experimental period, very few radioactive pulp cells were seen on or near the pulpal floor (Figure 11).

3. Root Canal Region:

By comparison with the rest of the pulp, radioactive cells were seldom seen in the root canal.

4. Apical Root Region:

Within the first two to eight hours after injection a few radioactive pulp nuclei were seen adjacent to Hertwig's diaphragm (Figures 12A, 12B). The mitotic activity at the root apex was increased gradually throughout the one hundred post injection hours of the experiment (Figures 12A, 12B). The pattern of radioactivity of the pulp cells at the root apex seemed to be opposite to the radioactivity of the pulp cells in the pulp horn, as the experimental period became greater.

The general results of the autoradiographic study are illustrated in Table I and Figure 13. It was found, however, that there was considerable variability in the radioactivity patterns of the pulp cells, even in the same tooth. In general, the root canal had the least radioactive pulp cells while in the regions of the horn or apical root and near Hertwig's epithelial sheath there were a greater number of radioactive cells.

B. Total Number of Pulp Cells and Its Relation to the Growth of the Pulp:

The counts were made through the entire normal pulp tissues (coronal and root portions) of 40 sections, each from two animals (at 2 hours and

96 hours after injection of tritiated thymidine). It was found that the normal pulp tissues of 60-day-old rats varied in cellular density (Table II). The lowest number of cells was 3009 per pulp section, and the highest number was 5339 as indicated in Table II. The increased number of cells between a 2 hour interval to 96 hour interval is 690. This result was evaluated by statistical analysis, which showed that the growth of pulp between 2 hours to 96 hours was probable at ten percent of significant level (Table III). Therefore, it is suggested that some growth of the pulp occurs in 60-day-old rats.

The greater cellular density was located both near the pulp horn, and the apical end of the root; the pulp cells were less dense throughout the remainder of the pulp.

C. Interpretation of the Frequency of Labeled Cells:

An attempt was made to determine the duplicating time of pulp cells. It was found that the percentage of labeled cells at 2 hours, post injection, gradually increased up to 60 hours. The percentage of labeled cells per pulp increased from the average of 7 cells to 15 cells within the range beginning at 22 and extending to 60 hours after injection of tritiated thymidine (Table I). After 60 hours, the percentage of labeled cells

declined until at 72 hours, it reached a constant level throughout the balance of the experimental period (Figure 14).

The duplicating time (the time at which the number of radioactive cells is doubled) occurred at sometime after 22 hours post injection of tritiated thymidine.

# CHAPTER V

# DISCUSSION

A. Radioactive Labeled Cells in Relation to the Growth of the Pulp Tissue:

In any tissue, large numbers of cells are produced and lost every day. Cell populations increase in number during periods of growth of an organism. At the same time, however, the rate of growth or proliferation varies in different parts of the organism (Langman, 1963). This rate is genetically determined in order to sustain the specific growth, and to replace lost cells. Thus, newly formed cells are needed to increase cell numbers in growth and replace the cells that are lost.

In the pulp tissue, this characteristic also holds true, giving this tissue a balanced cell turnover.

The radioactive tritiated thymidine labeled cells which are indicative of the DNA synthesis that precedes pulp cellular division, are the subject of attention in this study.

The radioactivity patterns in the pulp vary, as shown in Table I. The number of radioactive pulp horn cells is greater than in the other areas of the pulp at the beginning of the experimental period. This differed, however, from Pinson and associates' findings (1966) which

stated that the number of radioactive cells was highest at the root apex near the epithelial root sheath. Their study, however, used animals of various age groups. The large number of the horn cells seen in the present study is probably due to stimulation from the attrition of the teeth during mastication. As described in the results, labeled cells appeared first in the central area of the pulp horn. As the time after thymidine injection increased the position of the highest concentration of radioactive cells appeared to shift. Some appeared close to the odontoblastic layer, some appeared near the blood vessel wall, and others appeared in the tip of the horn or adjacent to the pulp chamber. It is possible that the labeled cells which appeared near the odontoblastic layer might be the precursors of odontoblasts; also those located near blood vessel walls, might be the precursors of the endothelial cells.

There are, however, radioactive cells throughout the entire pulp, the least number being found in the area of the root canal. The presence of labeled cells in the pulp could be explained by the hypothesis that since each cell has a limited life span, it must, therefore, be replaced by a newly formed cell in order to maintain the tissue.

Some radioactive cells were found concentrated in the root apex adjacent to Hertwig's epithelial root sheath, and a few were found near the floor of the pulp chamber. This might explain the source of cementoblasts, fibroblasts and osteoblasts as the molar erupts throughout the life span of the rat.

B. The Cell Density of the Pulp of 60-Day-Old Rats:

The cell density of the pulp tissue of the 60-day-old rats, two hours after injection of the tritiated thymidine averages 3901 cells per section, and at 100 hours post injection of the tritiated thymidine, the number increases to 4591 cells per section. This increased number is probably due to the multiplication of the cells in the pulp.

The average number of the pulp cells is 4246 cells per section. This, however, is greater than that reported in the recent study by Pinzon and associates (1966). They found the average number of the pulp cells in seven sections (2509 cells) less than the present study of forty sections; therefore, the result of the present study should be more reliable than the previous one.

The result of the statistical evaluation, using "t test" shows that, the evidence of growth of the molar pulps of 60-day-old rats has a ten

percent probability of significance level. It could be interpreted that the molar pulps are likely to be capable of growth. Therefore, the pulp cells must duplicate themselves in order to increase the cell population. However, the newly formed cells probably are needed not only for growth of the pulp itself but also for the replacement of the aging and degenerated cells.

The evaluation of the normal pulp tissues of the 60-day-old rats also suggests that during the period of time before the duplicating time ends, the areas of cellular density of the pulp varies directly as the labeled cell density. This can be explained simply as the labeled cells divide, they are paired side by side. Furthermore, there are more labeled cells in areas of greater cell density. However, after the cells divide, there must be either a migration of such cells and/or loss of labeled cells. This could explain the reduction of labeled cells seen in the pulp in the later hours and the loss of pairing of labeled cells.

C. Labeling Index:

This study has attempted to determine the labeling index in order to determine the duplicating time of the normal molar pulp tissues of the maxillary first molar of the female 60-day-old rats.

A labeled cell is a cell that takes up the radioactive thymidine at the time the radioactive thymidine is injected.

Prior to entering mitosis, the cell duplicates its DNA (Hughes 1958). It is during this stage that the cells which are actively synthesizing DNA are exposed to tritiated thymidine. This radioactive thymidine enters the DNA molecule in place of the naturally occurring thymine. This is because thymidine is an essentially specific precursor of thymine (Friedkin et al 1956). After that, the exposed cells become radioactive themselves, permitting one to study them, autoradiographically. All the cells, that take up injected tritiated thymidine, must be in a phase of DNA synthesis, but some might be at the beginning, some at the middle, the others still at the end of the phase. Therefore, the radioactive cells which appeared at the earliest experimental time were unevenly labeled.

Hughes, 1959 and Skongaard, 1964 stated that by two hours the injected thymidine probably should not be left in the circulation. Thus, the cells that are actively synthesizing DNA should pick up thymidine within the first two hours post injection of tritiated thymidine, and from then on, the number of labeled cells would be increased with increase of

time of sacrifice of the animal; this would be due to the cellular division of the labeled cell population.

There were, however, many cells which were in the period of DNA synthesis at the injection time that apparently could but did not take up thymidine; or some cells probably did take up thymidine, but in small amounts. These cells were not detectable. Therefore, the number of the labeled cells observed at any period does not represent one hundred percent of the actual number of labeled cells.

D. Duplicating Time of Pulp Cells:

The duplicating time occurred at sometime after twenty-two hours post injection of tritiated thymidine. Das (1963) also studied pulp tissue duplicating time but used a tissue culturing method and observed pulp cells doubling in number at sometime after forty-eight hours which fell in the range of this present study.

The average number of labeled cells per section at the duplicating time (from twenty-two to sixty hours) was fifteen. This number varied slightly for about thirty-eight hours, then it declined, and began to reach a constant level from seventy-two hours until the end of the experimental period. It should be mentioned, however, that during the period of thirty

to forty hours, the percentages of labeled cells were slightly decreased from 0.375 percent to 0.225 percent. The percentage then slightly increased to 0.275 percent at fifty hours. The labeled cell percentage eventually rose to the original of 0.375 percent.

Variations in the percentage of labeled cells might be due to various factors.

Because not all the cells that were actively synthesizing DNA were at the same stage of synthesis, they took up varying quantities of radioactive thymidine. Some cells, therefore, were clearly distinguishable as labeled cells but others had not enough radioactive granules within them to make them autoradiographically obvious and thus the number of labeled and obvious cells is less than should be.

Another factor that might reduce cell number (labeled) is the inhibition of cell division by the radioactive substance (tritium).

In addition, from a theoretical viewpoint, sectioning serially at five microns thickness should allow the most energetic beta particles (1.5 microns) to penetrate initially into the emulsion; however, in this study specimen section thickness was not always consistent. As a result the radioactive beta particles might not have penetrated uniformly and might not be detected.

Another factor is important in this study. Only one animal was utilized at each interval; therefore, it is possible that a natural variation exists in the potential for growth leading to variation in cell counts.

Finally, the loss of labeled cells following the termination of the duplicating time may be the result of death of cells due to radiation and/or the dilution of the labeled granules by succeeding cell division.

# CHAPTER VI

# SUMMARY AND CONCLUSIONS

## SUMMARY

Fifty female albino 60-day-old rats, were injected intraperitoneally with tritiated thymidine. At two-hour intervals over a period of one hundred hours, each injected animal was anesthetized and sacrificed. The maxillary molar regions were fixed immediately in ten percent buffered formalin, decalcified in formic-citric acid, dehydrated in ascending order of ethyl alcohol (75, 95 and 100%) and embedded in paraffin.

Mesio-distal sections were obtained through the maxillary first molar pulps. The histochemical staining was with Nuclear Fast Red and Indigo Carmine.

Autoradiographs were prepared as films adhered to the stained slides. Counts were made of those pulp cells labeled by uptake of the radioactive thymidine, excluding odontoblasts, blood cells and endothelial cells.

The percentage of labeled cells to nonlabeled cells of the pulp at each two-hour interval was calculated.

# CONCLUSIONS

1. As early as two hours after injection of radioactive thymidine, the radioactivity of the pulp tissue proper can be observed and this continues for at least one hundred hours.

2. The central area of the pulp horn and the area adjacent to Hertwig's epithelial root sheath have a greater incidence of DNA synthesis indicating a greater capability for cellular division than other areas of the pulp as indicated by the number of labeled cells.

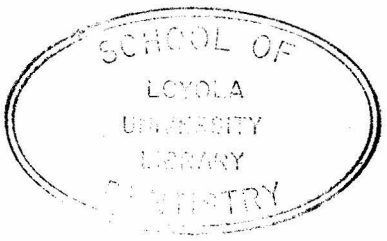
3. The least cellular division was located in the root canal region; however, a very few labeled cells were also observed in the region of the pulp floor.

4. The pulp at the root apex, adjacent to Hertwig's epithelial sheath, appeared to have a greater number of radioactive cells than the pulp in the horn region, as the experimental period increased.

5. The range of number of cells in the rat molar pulp is 3009 to 5337 cells.

6. The duplicating time of molar pulp tissue proper occurs after twenty-two hours.

7. The molar pulps of the 60-day-old rats are capable of growth, as it can be expressed in the term of probability as slightly greater than ten percent significant level.



# CHAPTER VII

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# CHAPTER VIII

## ILLUSTRATIONS

TABLE I

FREQUENCY DISTRIBUTION OF TRITIATED THYMIDINE LABELED CELLS IN THE RAT MOLAR PULP AT APPROXIMATELY 60 DAYS OLD

**(In calculating, the average number of pulp cells used was 4,000 instead of 4,246 cells)**

| Number Of Hours | Average Number Of Labeled Cells Per Section | Percentage Between Labeled Cells And Pulp Cells | Log Of Per Cent Between Labeled Cells and Pulp Cells |
|---|---|---|---|
| 2 to 10 | 7 | 0.175 | 0.2430 |
| 12 to 20 | 10 | 0.250 | 0.3979 |
| 22 to 30 | 15 | 0.375 | 0.5740 |
| 32 to 40 | 7 | 0.225 | 0.3522 |
| 42 to 50 | 11 | 0.275 | 0.4393 |
| 52 to 60 | 15 | 0.375 | 0.5740 |
| 62 to 70 | 11 | 0.325 | 0.5119 |
| 72 to 80 | 9 | 0.225 | 0.3522 |
| 82 to 90 | 9 | 0.225 | 0.3522 |
| 92 to 100 | 9 | 0.225 | 0.3522 |

TABLE II

AVERAGE NUMBER OF PULP CELLS PER SECTION IN A FIRST MAXILLARY MOLAR OF 60-DAY-OLD RATS

| Number Of Hours After Injection Of Tritiated Thymidine | Number Of Pulpal Cell Population | | | Average Of Pulpal Cell Population | |
|---|---|---|---|---|---|
| 2 | 3,841 | 4,362 | 3,952 | | |
| | 4,106 | 4,257 | 4,314 | | |
| | 4,795 | 3,822 | 3,691 | 3,901 | |
| | 3,703 | 3,919 | 3,511 | | |
| | 3,500 | 3,009 | 3,745 | | 4,246 |
| 96 | 5,112 | 5,116 | 4,001 | | |
| | 4,522 | 4,799 | 4,716 | | |
| | 5,269 | 4,353 | 5,195 | | |
| | 5,003 | 5,020 | 4,386 | | |
| | 4,857 | 4,130 | 4,425 | 4,591 | |
| | 4,162 | 4,719 | 4,435 | | |
| | 4,463 | 3,962 | 4,006 | | |
| | 3,975 | 4,340 | 5,337 | | |
| | 4,470 | | | | |

## TABLE III

DATA OF "T TEST" SHOWED THE PROBABILITY OF GROWTH IN THE MOLAR PULPS OF 60-DAY-OLD RATS

| | Mean Of Pulp Cells At 2-Hour Interval | Mean Of Pulp Cells At 96-Hour Interval |
|---|---|---|
| | 3,901 | 4,591 |
| $(\text{Standard Deviation})^2$ Or Variance | +898,575 | -724,819 |
| Standard Error Of Difference | 413.2 | |
| "T Test" | 1.67 ; P>10% | |

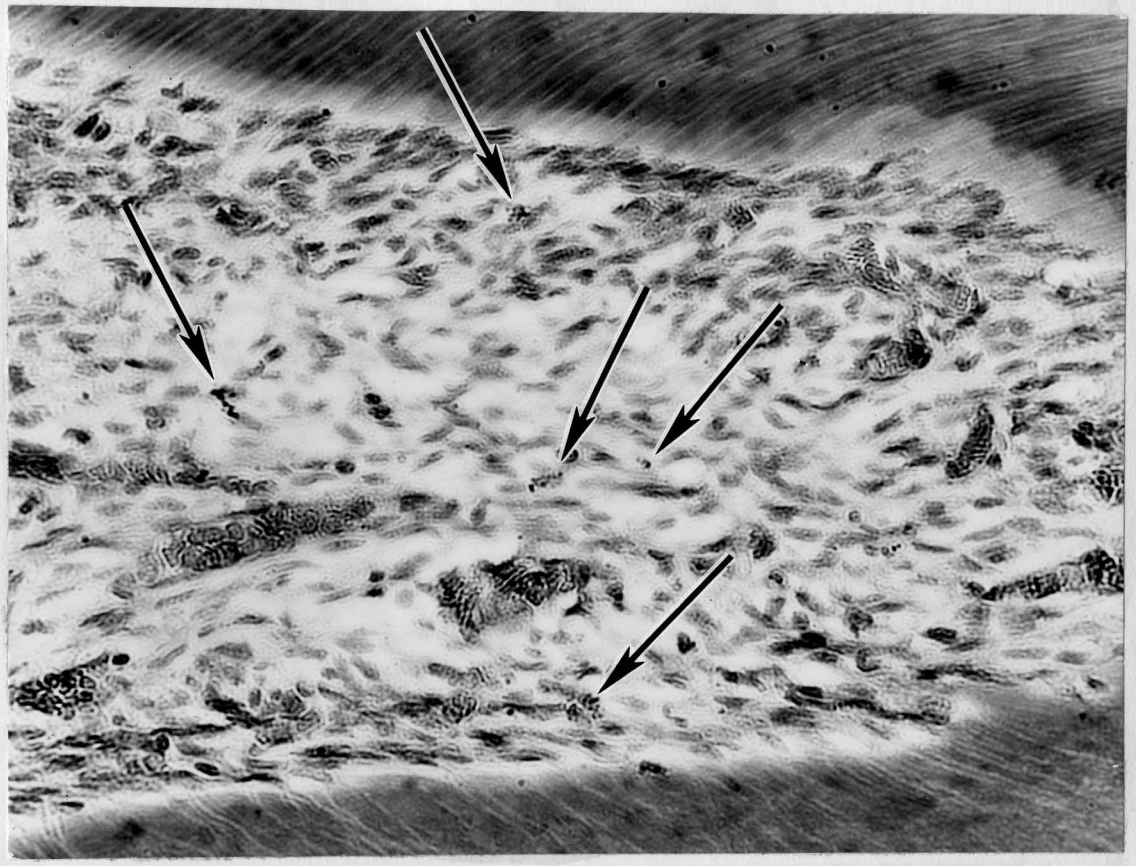

FIGURE 1

PHOTOMICROGRAPH SHOWING SILVER GRAINS OVER
THE NUCLEI AFTER BEING DEVELOPED WITH KODAK D-19

FIGURE 2A

PHOTOMICROGRAPH OF A MESIO-DISTAL CENTRAL SECTION
OF A MAXILLARY FIRST MOLAR,
TYPICALLY CHOSEN IN THIS STUDY

MAGNIFICATION X 20

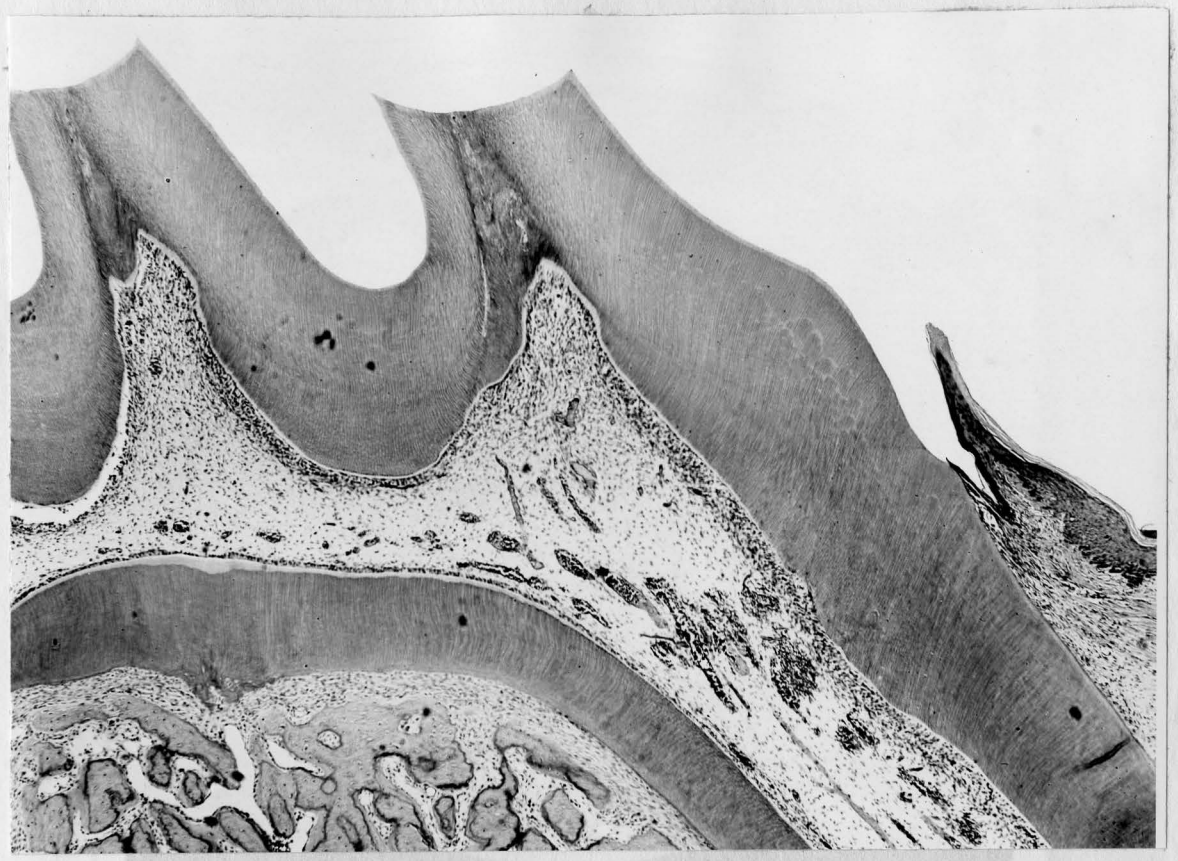

FIGURE 2B

PHOTOMICROGRAPH OF A MESIO-DISTAL CENTRAL SECTION
OF A MAXILLARY FIRST MOLAR,
TYPICALLY CHOSEN IN THIS STUDY

MAGNIFICATION X 48
(ENLARGED FROM THE VIEW INDICATION IN FIGURE 2A)

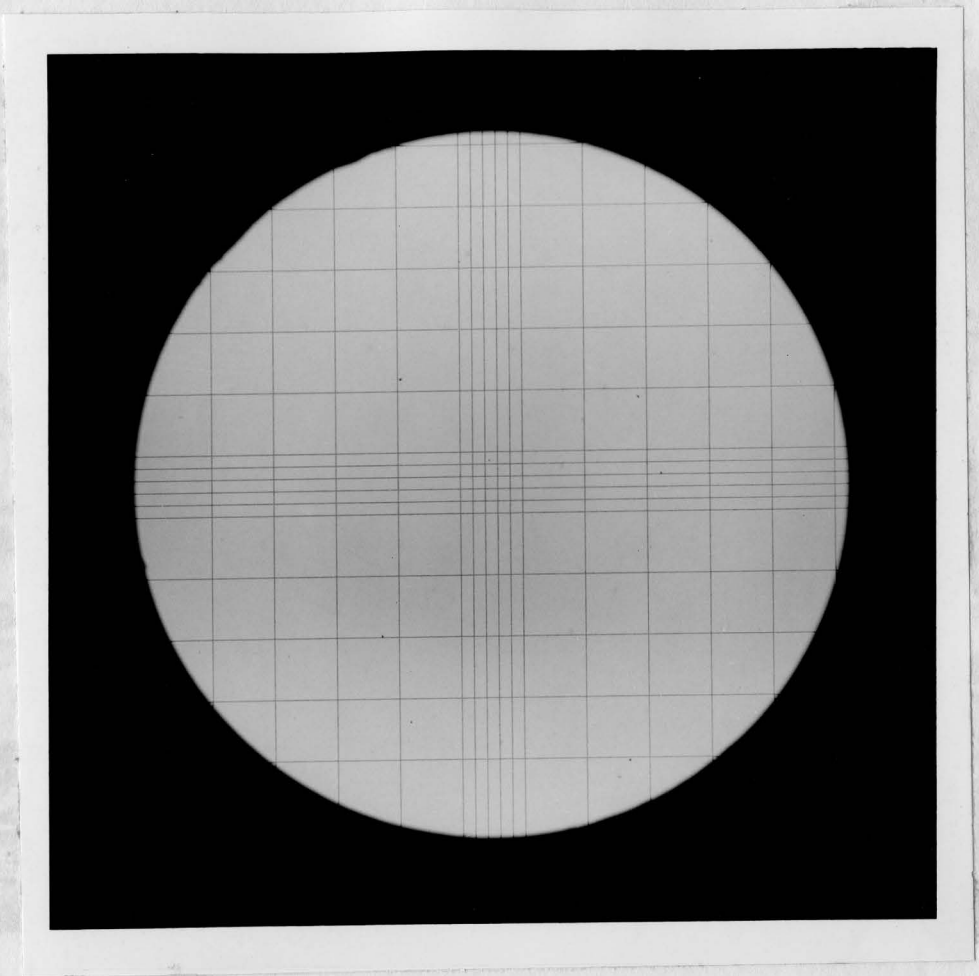

FIGURE 3

PHOTOGRAPH SHOWING THE SQUARES IN THE WHIPPLE DISK WHICH IS USED AS A GUIDE IN CELL COUNTS

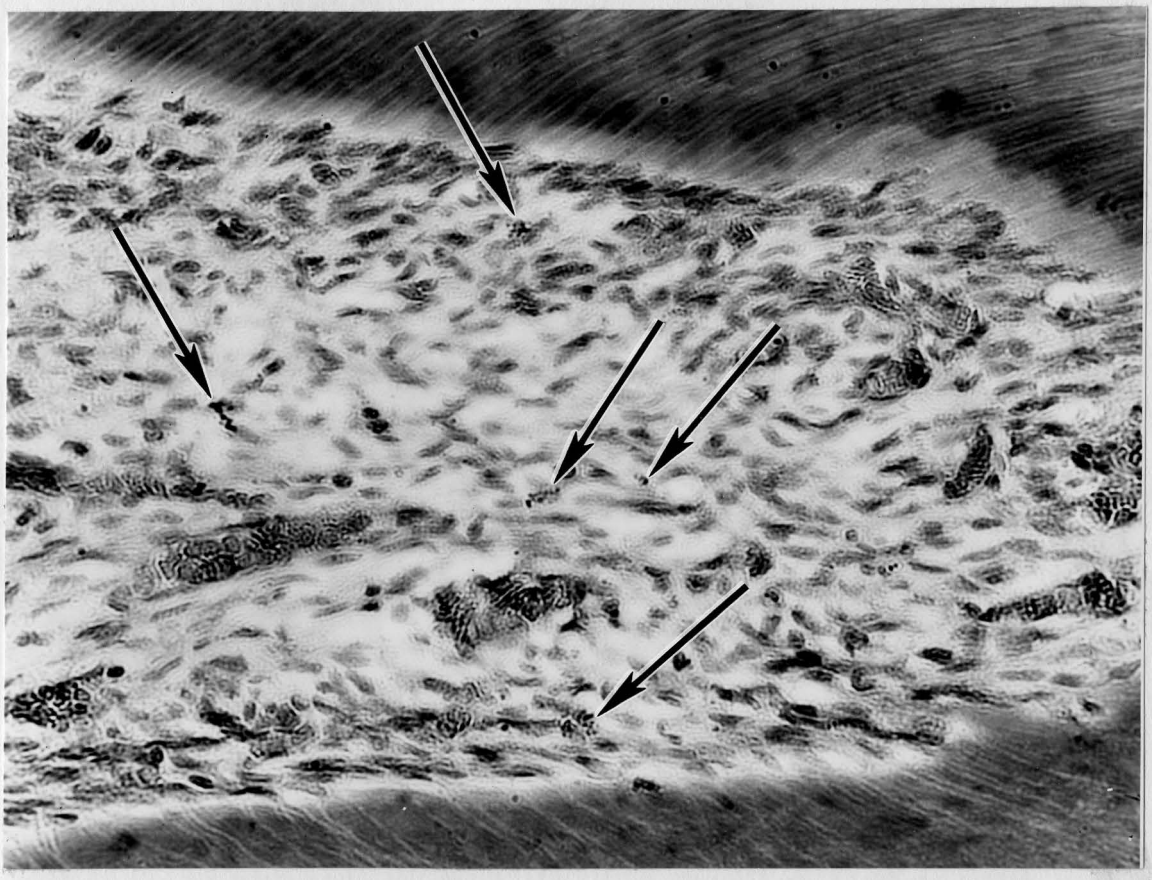

FIGURE 4

PHOTOMICROGRAPH SHOWING A GOOD EXAMPLE OF LABELING AND THE LEAST RADIATION BACKGROUND IN SECTIONS USED IN THIS STUDY

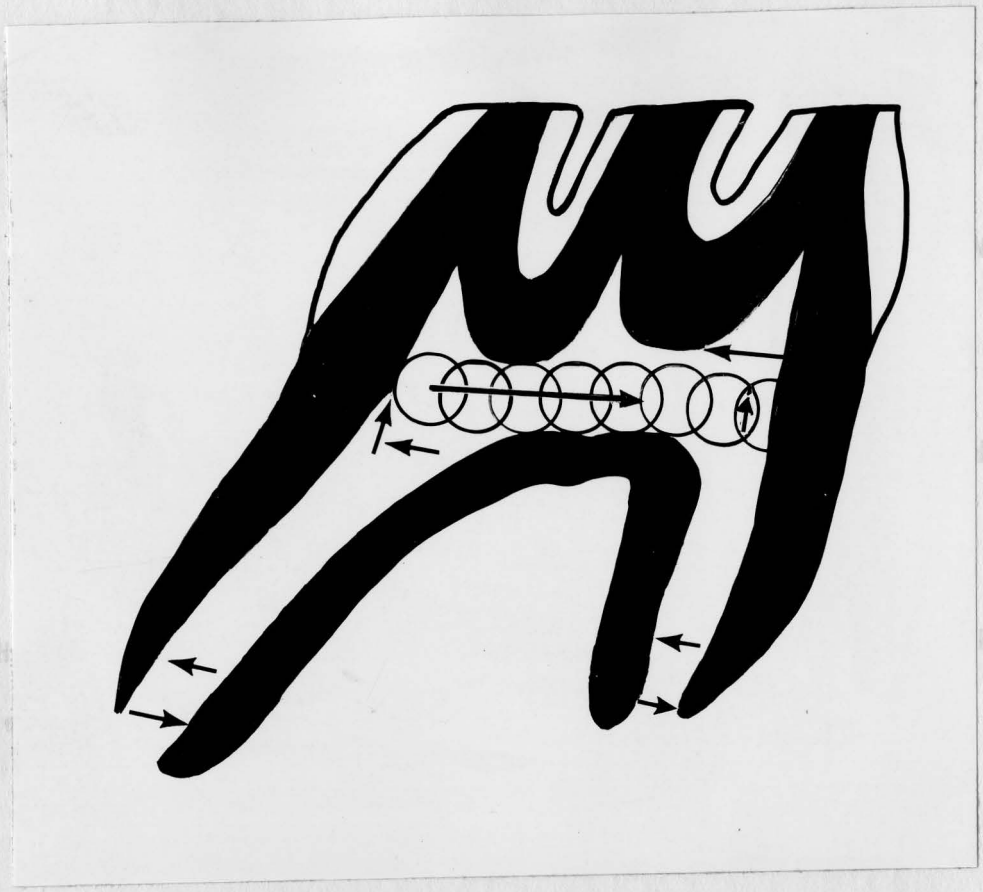

FIGURE 5

DIAGRAM SHOWING THE DIRECTION OF MOVEMENT (ARROWS) OF THE SLIDE, FOLLOWED IN MAKING COUNTS OF THE LABELED CELLS AND NONLABELED PULP CELLS

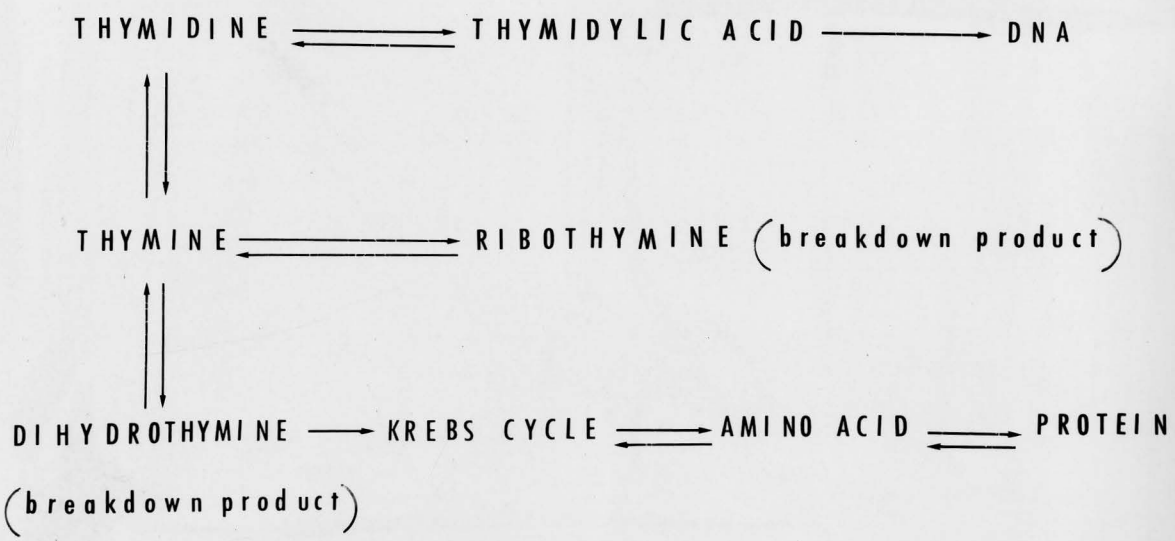


**FIGURE 6**

**DIAGRAM (AFTER POTTER, 1959) SHOWING THE ALTERNATIVE PATHWAYS OF TRITIATED THYMIDINE METABOLISM**

**THE NAMES USED HERE FOR THE RIBOSE DERIVATIVES OF THYMIDINE WERE BEING USED BY GRIFFIN, TODD AND RICH 1958**

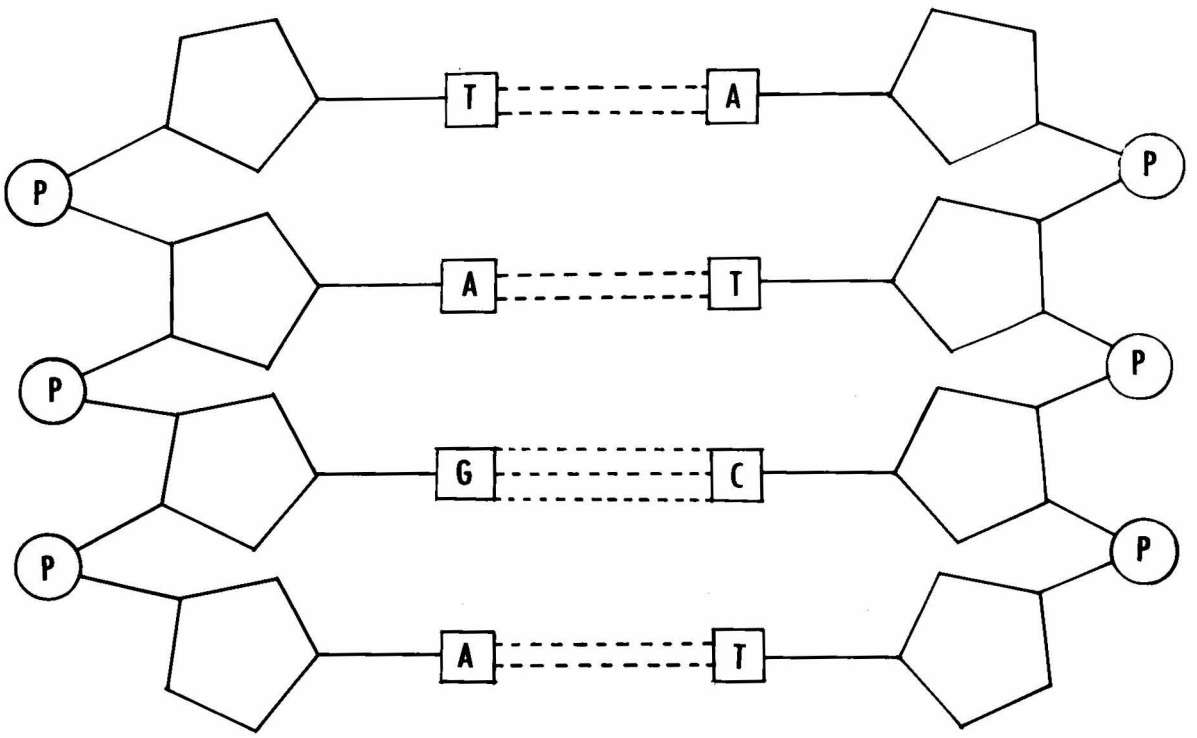


**FIGURE 7**

**SCHEMATIC REPRESENTATION OF THE STRUCTURAL FORMULA OF DNA BY WATSON AND CRICK, 1953**

P = PHOSPHATE

⬠ = DEOXYRIBOSE

A = ADENINE (PURINE BASE)

G = GUANINE (PURINE BASE)

T = THYMINE (PYRIMIDINE BASE)

C = CYTOSINE (PYRIMIDINE BASE)

FIGURE 8

AUTORADIOGRAPH OF THE HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, TWO-HOURS AFTER INJECTION OF TRITIATED THYMIDINE

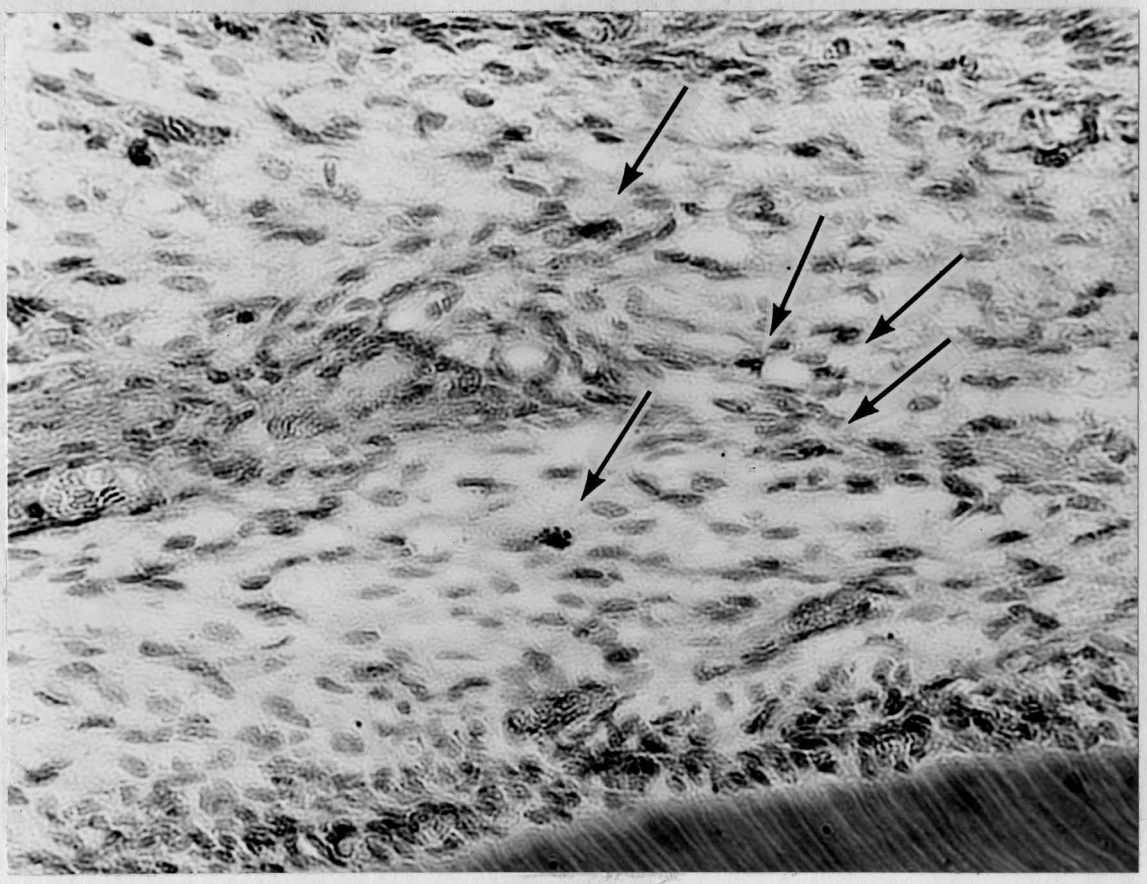

FIGURE 9

AUTORADIOGRAPH OF THE HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT AT FOUR-HOURS AFTER INJECTION OF TRITIATED THYMIDINE

NOTE THE LOCATION OF THE RADIOACTIVE CELLS

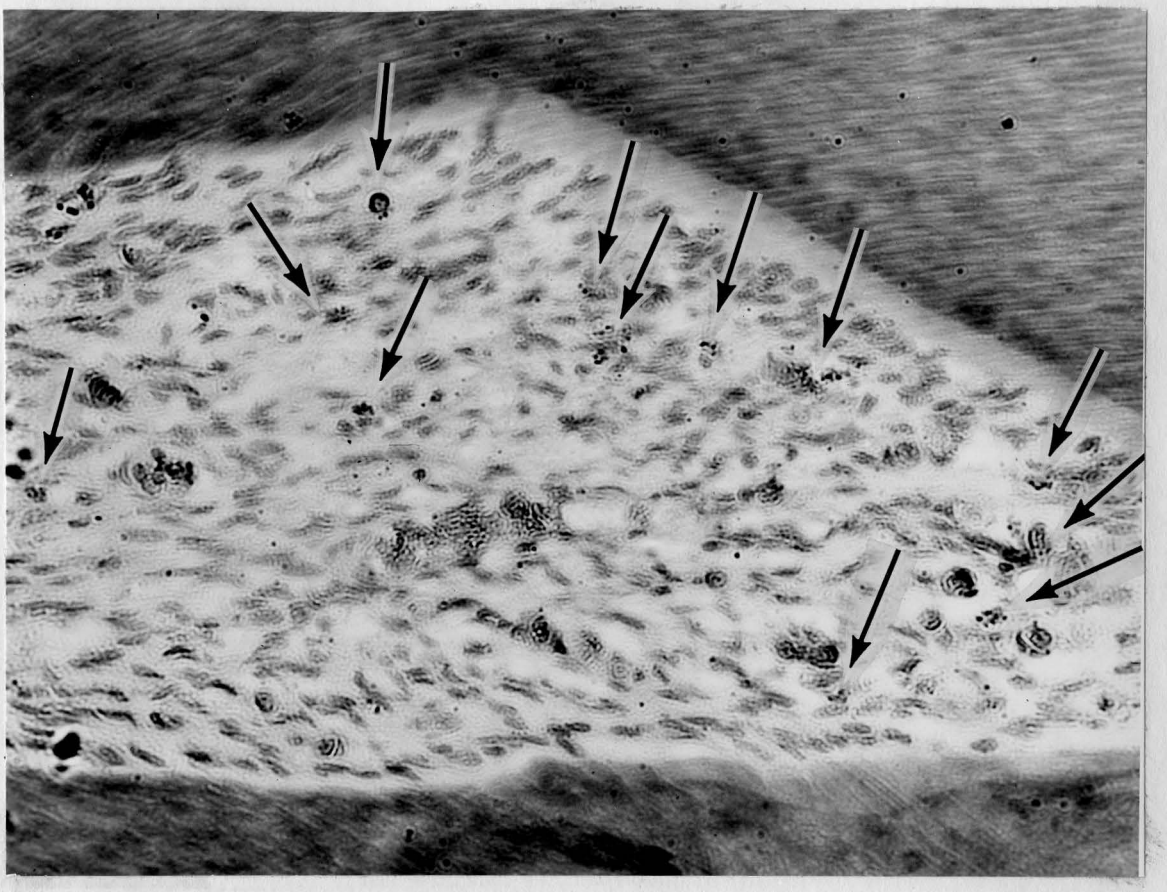

FIGURE 10

AUTORADIOGRAPH OF THE HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, AT TWENTY-EIGHT HOURS AFTER INJECTION OF TRITIATED THYMIDINE

NOTE BOTH THE INCREASED NUMBER OF LABELED DISTRIBUTION AS COMPARED WITH BOTH THE TWO AND TWENTY-FOUR HOUR SPECIMENS

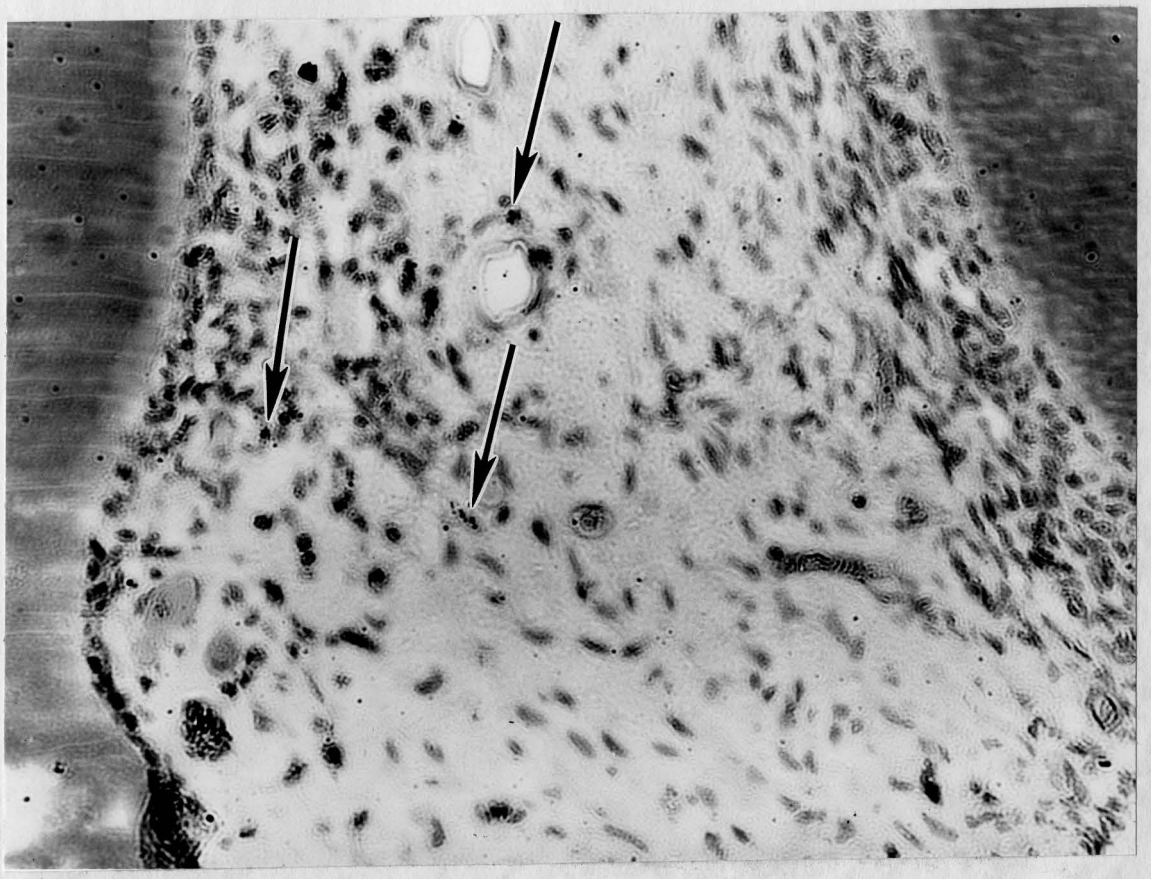

FIGURE 11

AUTORADIOGRAPH OF THE HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, AT TWENTY-EIGHT HOURS AFTER INJECTION OF TRITIATED THYMIDINE

NOTE THE LOCATION OF RADIOACTIVE CELLS ON THE PULP FLOOR

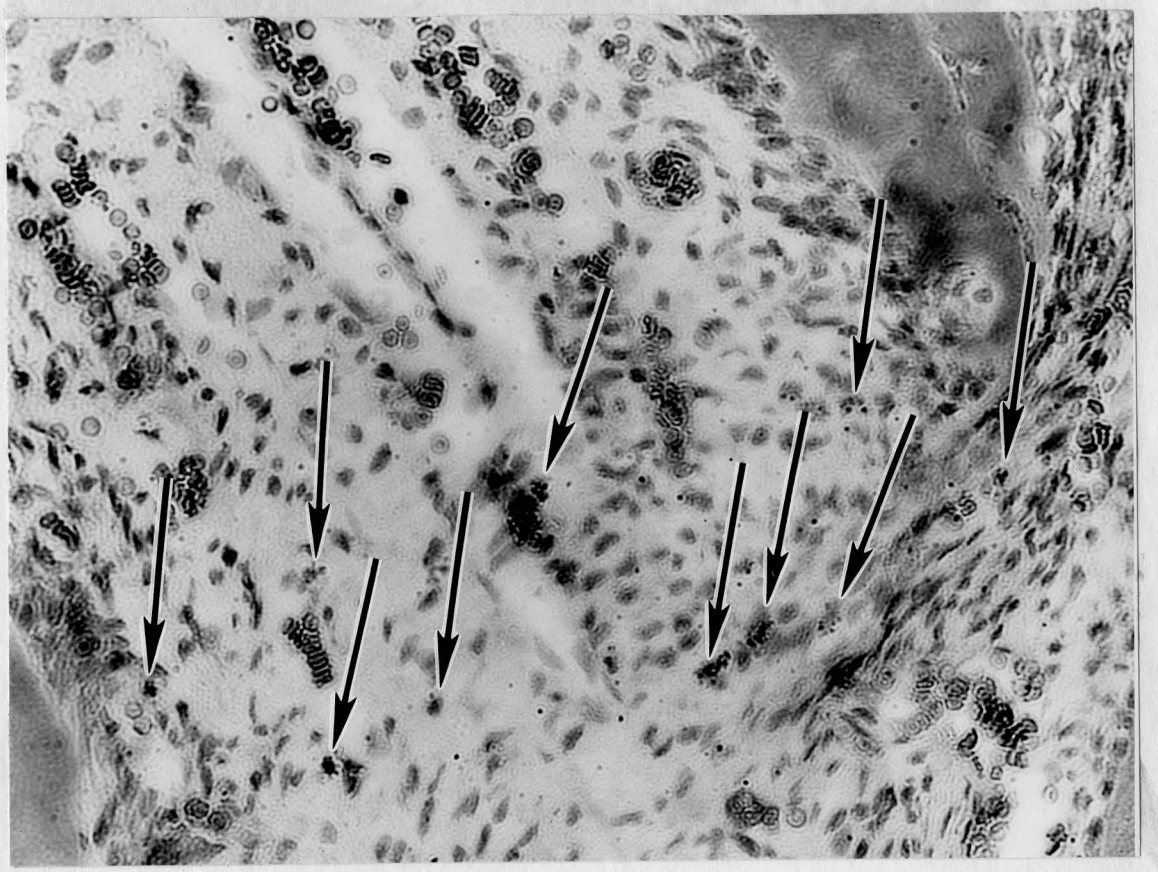

FIGURE 12A

AUTORADIOGRAPH OF THE HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, AT SEVENTY-HOURS AND AFTER INJECTION OF TRITIATED THYMIDINE

NOTE THE LOCATION OF RADIOACTIVE CELLS OF THE APICAL PULP

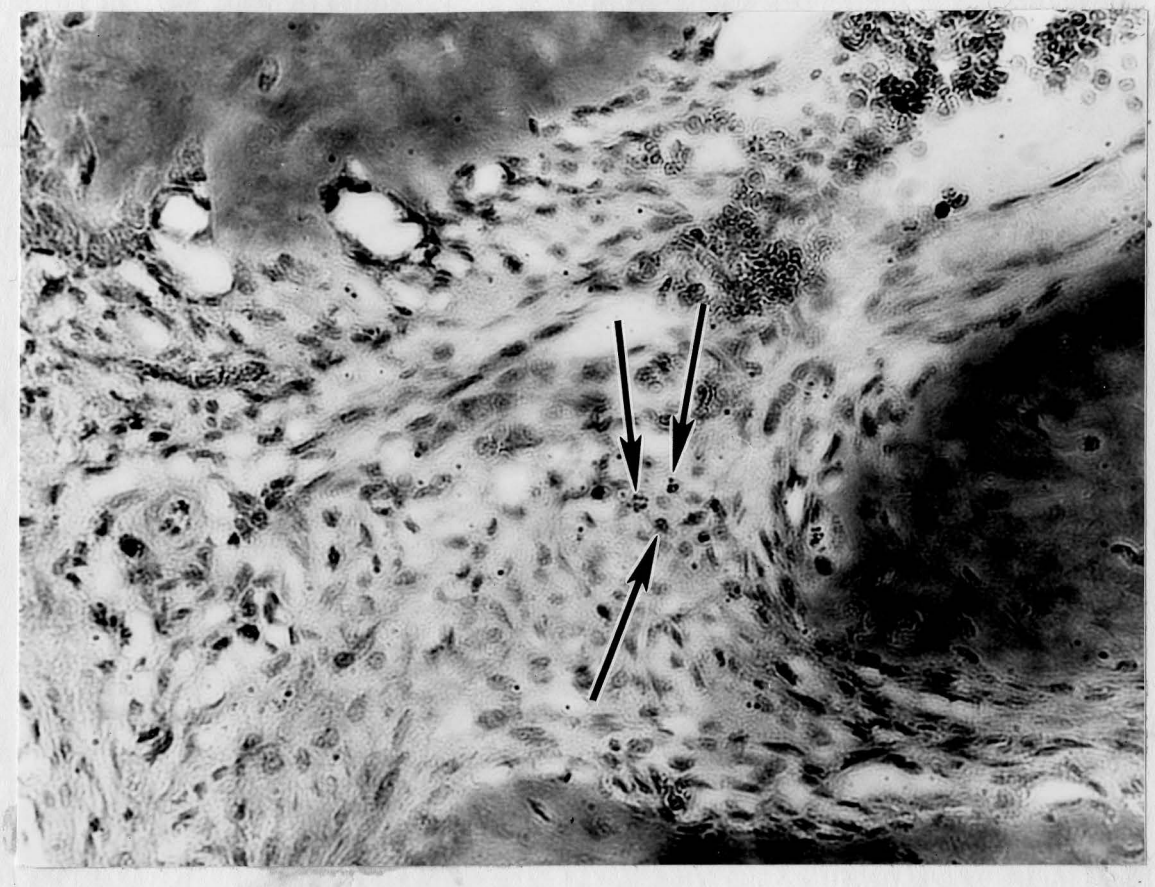

FIGURE 12B

AUTORADIOGRAPH OF THE HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, AT NINETY-SIX HOURS AFTER INJECTION OF TRITIATED THYMIDINE

NOTE THE LOCATION OF RADIOACTIVE CELLS OF THE APICAL PULP

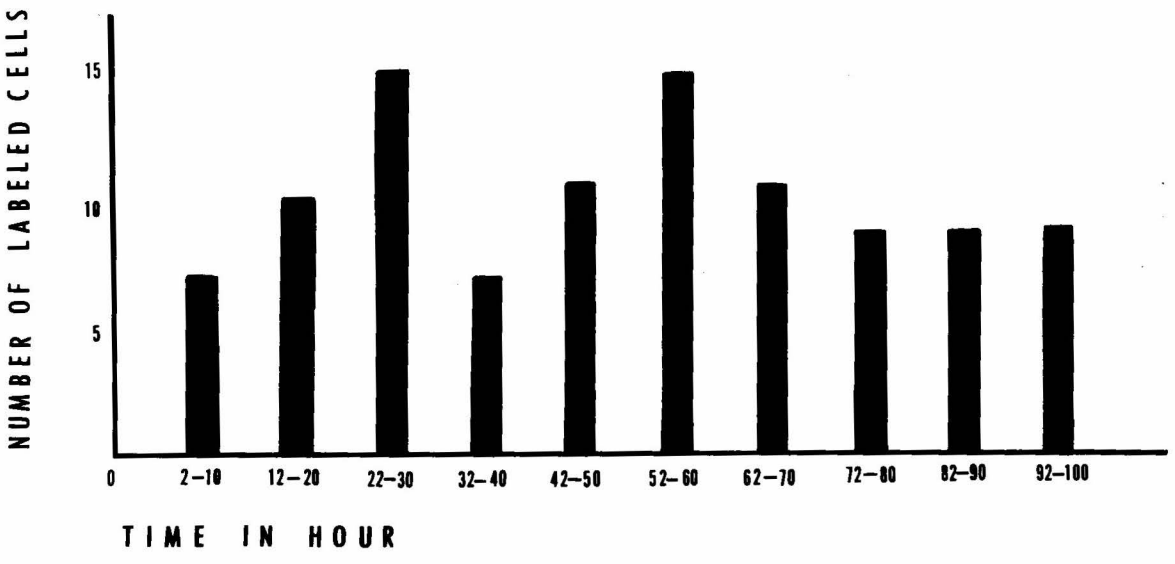


FIGURE 13

DIAGRAM SHOWING THE TOTAL COUNTS
OF RADIOACTIVE NUCLEI
DISTRIBUTED IN THE PULP TISSUE PROPER

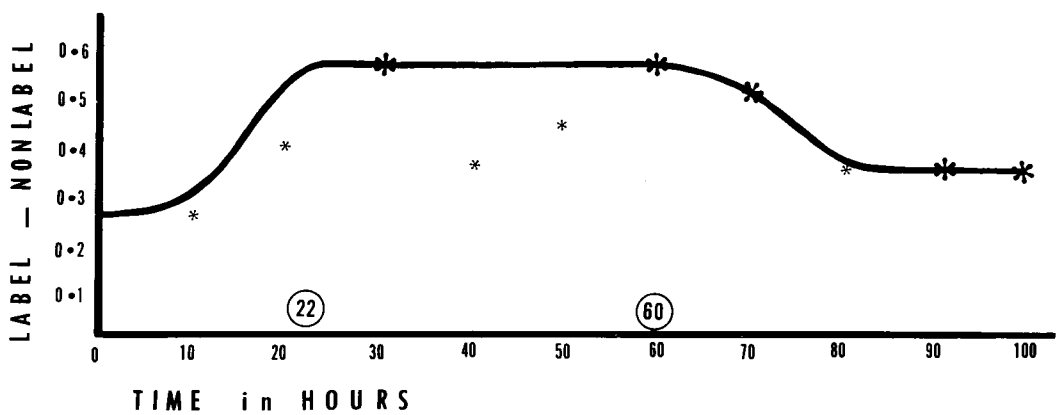


FIGURE 14

GRAPH SHOWING THE DUPLICATING TIME

FIGURE 15

AUTORADIOGRAPH OF THE HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, AT SIXTY-HOURS AFTER INJECTION OF TRITIATED THYMIDINE, SHOWING THE LABELED CELLS NEAR CEMENTUM AT APEX OF ROOT

FIGURE 16

PHOTOMICROGRAPH OF HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, AT TWENTY-FOUR HOURS AFTER INJECTION OF TRITIATED THYMIDINE

NOTE BOTH THE HEAVILY LABELED CELLS AND LIGHTLY LABELED CELLS

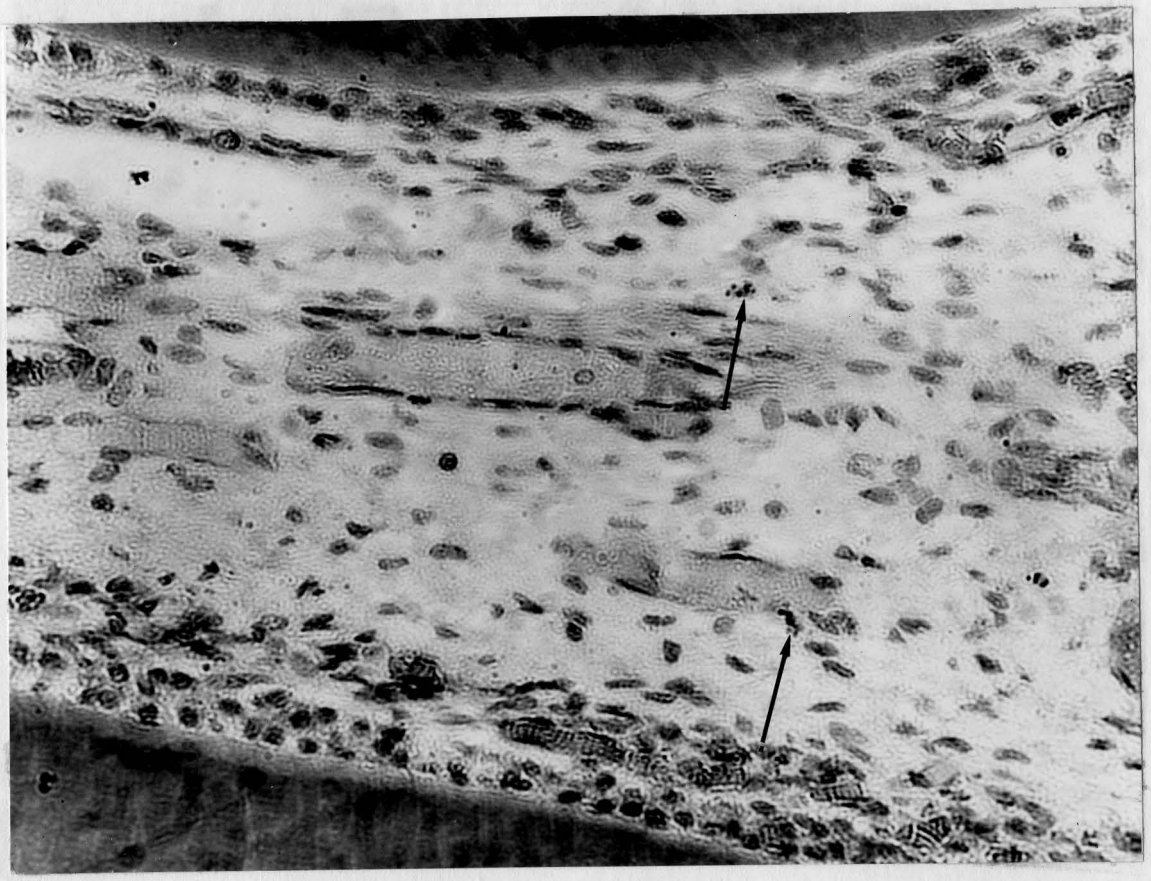

FIGURE 17

PHOTOMICROGRAPH OF HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, AT TWENTY HOURS AFTER INJECTION OF TRITIATED THYMIDINE

NOTE THE LOCATION OF RADIOACTIVE CELLS OF THE ROOT CANAL REGION

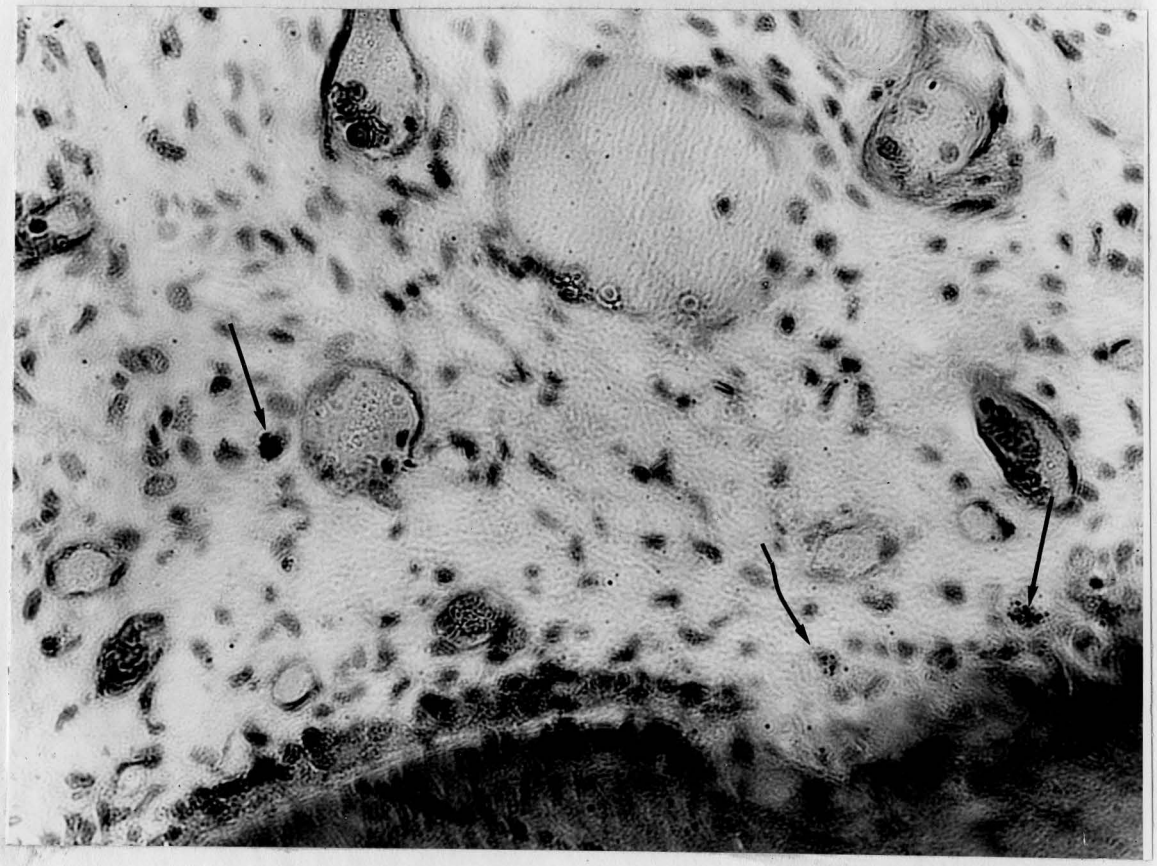

FIGURE 18

AUTORADIOGRAPH OF THE HISTOLOGICAL SECTION OF A MAXILLARY FIRST MOLAR, OF A 60-DAY-OLD RAT, AT TWO HOURS POST INJECTION OF TRITIATED THYMIDINE SHOWING VARIATION OF TAKING UP THE RADIOACTIVE THYMIDINE

FIGURE 19

AUTORADIOGRAPH SHOWING THE RADIOACTIVE CELLS AT THE FIRST TWO HOURS AND THE ONE HUNDRED HOURS POST INJECTION (MEANS A NUMBER OF SILVER GRAINS)

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1968.

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APPROVAL SHEET

The thesis submitted by Dr. Nipavann Taiyong has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is, therefore, accepted in partial fulfillment of the requirements for the Degree of Master of Science.

DATE: 5-24-68

Marvin Kozlov, D. D. S., M. S.

Marvin Kozlov DDS, M.S.
Signature of Advisor